Paraovarian Cysts Associated With Prenatal Diethylstilbestrol Exposure

Comparison of the Human With a Mouse Model

A. F. HANEY, RETHA R. NEWBOLD, BERNARD F. FETTER, and JOHN A. McLACHLAN

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, and the Department of Pathology, Duke University Medical Center, Durham, North Carolina, and the Developmental Endocrinology and Pharmacology Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina

The association of prenatal exposure to diethylstilbestrol (DES) and abnormalities in structures of müllerian (paramesonephric) origin has been well documented. In a murine model, exposure to DES *in utero* results in persistent mesonephric remnants in adult female mice. Six women exposed prenatally to DES had paraovarian cysts excised during routine gynecologic surgery; and in 4, histologic abnormalities were observed, including thickened fibromuscular walls with tall columnar epithelium in a papillary or pseudoglandular configuration. Four of 25 nonexposed and 8 of 9 DES-exposed infertile women undergoing surgery for infertility had paraovarian cysts, and the difference was statistically significant ($P < 0.02$). These findings raise the possibility that structures derived from the mesonephric ducts or tubules may also be affected in women exposed *in utero* to DES. (Am J Pathol 1986, 124:405–411)

CONCERNS regarding prenatal exposure to diethylstilbestrol (DES) in women have primarily focused on the structures derived from the müllerian (paramesonephric) ducts, with abnormalities being observed in the upper vagina,[1] cervix,[1-3] uterus,[2,4] and fallopian tubes.[5] Although DES seems to have a profound effect on the müllerian duct system, its effect on the mesonephric duct system has not been documented. Studies in our laboratory have focused on developing an animal model for further investigation of the effects of prenatal DES exposure. The CD-1 outbred mouse is an appropriate experimental model that demonstrates many of the benign and maligant changes seen in DES-exposed women.[6,7] Using this animal, we have shown that prenatal treatment with DES results in alterations in structures of mesonephric origin in female offspring in addition to changes in müllerian-derived tissues.[6,7] These results confirm animal studies dating back to the 1930s that have described proliferative changes in mesonephric tissues after DES administration.[8]

Although, to date, there have been no clinical reports suggesting that prenatal DES exposure is associated with histologic abnormalities of the mesonephric system in women, this report describes 6 women who had paraovarian cysts of probable mesonephric origin excised. Four of the 6 women had cysts with unusual histologic features, compared with paraovarian cysts from women without prenatal DES exposure. Mice prenatally exposed to DES developed prominent cystic structures of mesonephric origin. Similarly, the frequency of paraovarian cysts was increased in women exposed prenatally to DES in an infertile population undergoing gynecologic surgery. These observations raise the

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Address reprint requests to A. F. Haney, MD, Box 2971, Duke University Medical Center, Durham, NC 27710.

possibility that, as in the mouse, DES may induce prominent paraovarian cysts with abnormal histologic features in women.

Materials and Methods

The Murine Model: Treatment Schedule and Histologic Preparation

Outbred CD-1 mice (CrL: CD-1 [ICR] BR) were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. Females at approximately 6–8 weeks of age were mated with males of the same strain in the NIEHS animal facility, and detection of a vaginal plug was considered Day 0 of pregnancy. All animals were provided with hardwood bedding, fresh water, and NIH 31 laboratory mouse chow *ad libitum*.

DES (Sigma Chemical Co., St. Louis, Mo) was dissolved in corn oil and administered as a subcutaneous injection to pregnant females on Days 9–16 of gestation at a dose of 100 μg/kg (DES-100). Pregnant females exposed to DES and matched untreated controls delivered their young spontaneously or were delivered by cesarean section on Day 19 of pregnancy. Litter sizes were randomly standardized to 8 offspring per mother. At 25 days of age, offspring were weaned and housed 5 per cage. Reproductive tract tissues, including ovaries, from 8-month control and DES-100 animals were excised, fixed in 10% buffered formalin, dehydrated, embedded in paraffin blocks, serially sectioned at 6 μ, and stained with hematoxylin and eosin (H&E).

Clinical Features

All 6 women who had paraovarian cysts excised were known to have been exposed to DES in the first trimester of pregnancy. Their clinical data are presented in Table 1. The histologic specimens were excised at the time of surgery, fixed in 10% buffered formalin, embedded in paraffin blocks, sectioned at 6 μ, and stained with H&E for routine histologic examination. Adjacent sections were stained for the presence of glycogen with the Schiff stain before and after digestion by diastase.

To evaluate the frequency of paraovarian cysts with prenatal DES exposure, we combined the 6 women with paraovarian cysts excised with 3 additional women with prenatal DES exposure who underwent laparoscopies for infertility (Table 1) and compared them with 25 consecutive nonexposed women undergoing surgery for infertility. Only patients with pelvic anatomy that could be directly visualized were included. One woman with prenatal DES exposure and 3 control subjects were excluded because they had such severe pelvic inflammatory disease that the presence of paraovarian cysts could not be ascertained because of extensive pelvic adhesions. The Fisher's Exact test for qualitative variables was utilized for statistical analysis.

Results

Murine Disease

Figure 1 is a photomicrograph of an animal included in tabular form in a study of the ovaries and oviducts

Table 1—Clinical Findings in the DES-Exposed Women

			Anatomic abnormalities*			
	Age	Parity	Vagina	Cervix	Uterus	Other abnormalities
Paraovarian cysts excised						
1	29	0	–	+	ND	Hemorrhagic paraovarian cyst
2	27	0	+	+	+	Pelvic adhesions
3	30	0	+	+	+	Uterotubal junction obstruction, endometriosis
4	32 (2 untreated ectopic pregnancies)	2-2-0	–	–	+	Missing segments of both fallopian tubes[28]
5	33	0	–	+	+	Endometriosis
6	28 (2 spontaneous abortions and a term delivery)	3-2-1	+	–	+	Endometriosis
Laparoscopy only						
7	27	0	+	+	+	Endometriosis, pelvic adhesions
8	34	0	–	+	+	Endometriosis, pelvic adhesions
9	29	0	–	Cervical stenosis	+	Endometriosis, pelvic adhesions

* The anatomic abnormalities were defined as follows: vagina, adenosis; cervix, any of the typical DES-associated anomalies, such as a cervical hood, a cockscomb deformity, a cervical collar, a pseudopolyp, or a marked eversion of columnar epithelium on the exocervix; uterus, an irregular, small, T-shaped uterine cavity with a narrow endocervical canal as delineated by hysterosalpingography.

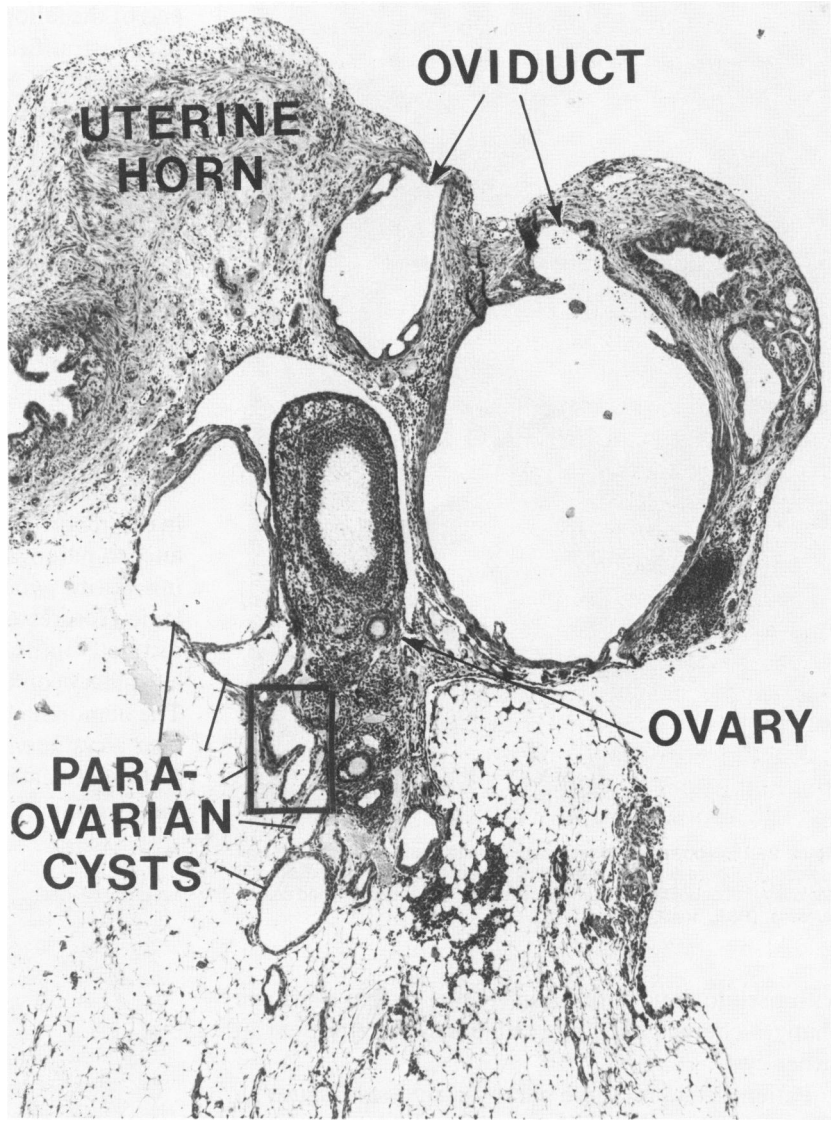

Figure 1—Low-power photomicrograph of the paraovarian cysts in a mouse exposed prenatally to DES. The overall relationship of the uterine horn, oviduct, ovary, and paraovarian cysts are shown. Note that these changes have previously been reported to be associated with prenatal DES exposure in this animal model.[8] The thin-walled paraovarian cysts in the lower portion of the figure are near the hilus of the ovary and are of mesonephric tubule origin. (H&E, ×40)

in mice after prenatal DES exposure.[7] This photomicrograph shows cystic paraovarian structures between the ovary and oviduct. The highest concentration of cysts in this mouse is in the region of the ovarian hilus. Serial sections demonstrate that these cysts are of mesonephric origin. Histologic variation is apparent in these cysts. The cyst in the upper portion of the photograph is surrounded by a layer of muscle, which is consistent with mesonephric origin, whereas the thin-walled cysts located in the lower portion of the figure have no muscle present. These cysts are of mesonephric tubule origin. The epithelial cells are cuboidal or columnar and sometimes have cilia (Figure 2). The nuclei are ovoid and centrally located. The epithelium in the lower cysts also is cuboidal to columnar. Some are ciliated, whereas others are vacuolated, with pale cytoplasm. Mesonephric cysts of this magnitude have never been observed in our control series of animals.

Human Disease

Patient 1 had a 5-cm hemorrhagic cyst in the expected location of paraovarian cysts (Figure 3). Microscopically, this lesion resembled a cystadenofibroma. The epithelium was tall columnar, with a mixture of mucus-secreting, ciliated, and intercalary cells. There was an overall papillary configuration with a dense stroma composed of spindle-shaped cells. Psammoma bodies were present. The second cyst was a typical-appearing paraovarian cyst on gross examination. Microscopically, it had a thickened fibromuscular wall, a papillary configuration, and pseudostratified columnar epithelium

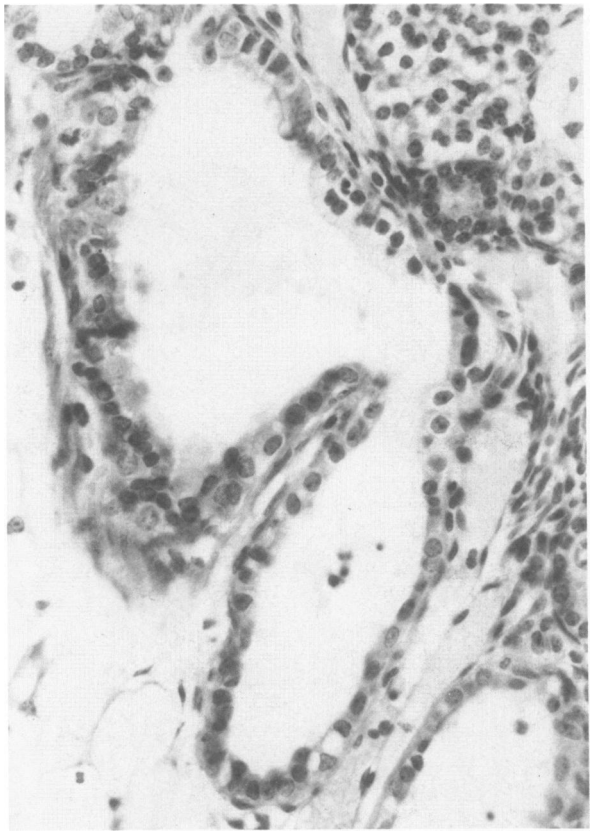

Figure 2—High-power photomicrograph of the murine paraovarian cyst depicted in Figure 5 (**Inset**). Note that the epithelium is composed of a single layer of cuboidal or columnar cells, with occasional ciliated columnar cells. (H&E, ×400)

with pseudogland formation (Figure 4). No cytologic changes characteristic of malignancy were present in either cyst.

Patient 2 had multiple paraovarian cysts (Figure 5). The arrangement is similar to the multiple cysts in the murine model shown in Figure 1. The histologic appearance of one of the cysts is shown in Figure 6A and is typical of paraovarian cysts in the nonexposed population. It revealed a thin-walled cyst with a delicate stroma. There was a single layer of cuboidal epithelial cells with an occasional ciliated cell. By contrast, an adjacent cyst (Figure 6B) was similar in appearance to the nonhemorrhagic paraovarian cyst in Patient 1. The epithelium was pseudostratified, with a convoluted surface, and the cyst wall had a thick fibromuscular layer. There was no cytologic evidence suggesting malignant change.

Patients 3 and 4 had multiple paraovarian cysts near the fimbriated end of the fallopian tube arising from within the mesosalpinx. Histologically, these were thin-walled cysts lined by a single layer of cuboidal cells similar to the cyst from Patient 2 presented in Figure 6A.

Patients 5 and 6 had multiple paraovarian cysts arising from within the mesosalpinx near the fimbriated end of the fallopian tube. In both patients the cysts had pseudostratified columnar epithelium in a papillary configuration with pseudogland formation similar to the histology of the nonhemorrhagic cyst in Patient 1 (Figure 4).

Eight of the 9 women with prenatal DES exposure whose pelvic anatomy could be adequately visualized had identifiable paraovarian cysts near the fimbriated end of the fallopian tube on at least one side. Four of the 25 women without DES exposure had paraovarian cysts identified. The difference was significant at the $P < 0.02$ level.

Discussion

The paraovarian cysts in the 6 DES-exposed women in this report were similar in location and gross appearance to paraovarian cysts frequently encountered during routine gynecologic surgery. By contrast, the histologic features of the cysts in 4 of the 6 women were distinctly unusual with pseudostratified epithelium in a papillary configuration and a thickened muscular wall. The hemorrhagic paraovarian cyst in Patient 1 resembled a cystadenofibroma and may be unrelated to the histologic features in the other patients or the other paraovarian cyst in Patient 1. Paraovarian tumors of

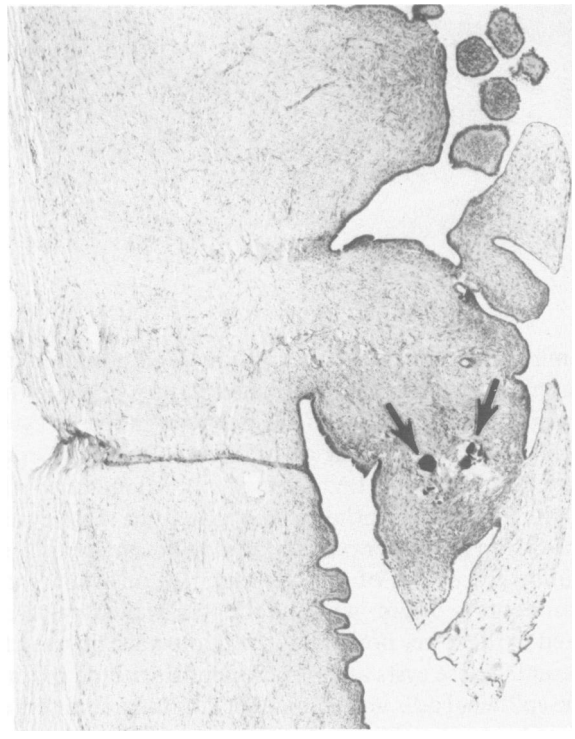

Figure 3—Photomicrograph of the hemorrhagic paraovarian cyst from Patient 1. Paraovarian papillary serous cyst with a dense fibromuscular wall in which psammoma bodies were present (*arrows*) and lined by a tall columnar epithelium. (H&E, ×40)

probable mesonephric origin have been noted previously without specific reference to DES exposure.[9] A potential relationship between prenatal DES exposure and histologic changes in paraovarian cysts deserves further inquiry in view of the profound effects of DES on the developing müllerian and mesonephric ducts.[7,10]

After the report by Herbst and colleagues describing a previously rare clear-cell vaginal adenocarcinoma associated with prenatal exposure to DES,[11] concern focused on the neoplastic potential of vaginal adenosis. Although the risk of malignancy was found to be relatively low, it became apparent that there were reproductive problems associated with prenatal DES exposure. Anatomic deformities have been observed in structures of müllerian (paramesonephric) origin, including the cervix,[1-3] uterus,[2,4] and fallopian tubes,[5] in exposed women. Controlled and uncontrolled studies suggest increased rates of ectopic pregnancy,[12-17] spontaneous abortion,[12,14,15,18] and premature labor.[12,13,15,17] Although the exact mechanisms of these reproductive problems are not known, a correlation with the anatomic abnormalities observed in the müllerian-derived tissues has been noted.[14,15]

Despite well-documented effects on the developing müllerian ducts, the impact of DES on the developing mesonephric system is less clear. It has long been observed in animal models that proliferative changes occur in the mesonephric ducts with prenatal exposure to DES.[8] In male offspring of both humans[19] and experimental animals,[20] structures derived from both the müllerian and mesonephric systems are altered by prenatal DES. Prior to this report, there was no clinical data suggesting a change in the incidence or histologic appearance of mesonephric remnants in DES-exposed women. Furthermore, no clinical problems attributable to paraovarian cysts in the DES-exposed population have been noted. This report describes histologic abnormalities in paraovarian cysts from 4 of 6 women exposed to DES *in utero*. Similar proliferative changes in the mesonephric system and intra- and paraovarian cysts from mesonephric remnants associated with prenatal DES administration have previously been reported in mice.[6,7] Studies from Vannier and Raynoud's laboratory have also reported persistent mesonephric ducts in the adult female rat with prenatal exposure to estrogenic compounds.[21] Robboy et al described actively growing the mesonephric type of epithelium in an *in vivo* model using human tissues derived from fetuses and transplanted into DES-treated mice.[22] These data together raise the possibility that the histologic abnor-

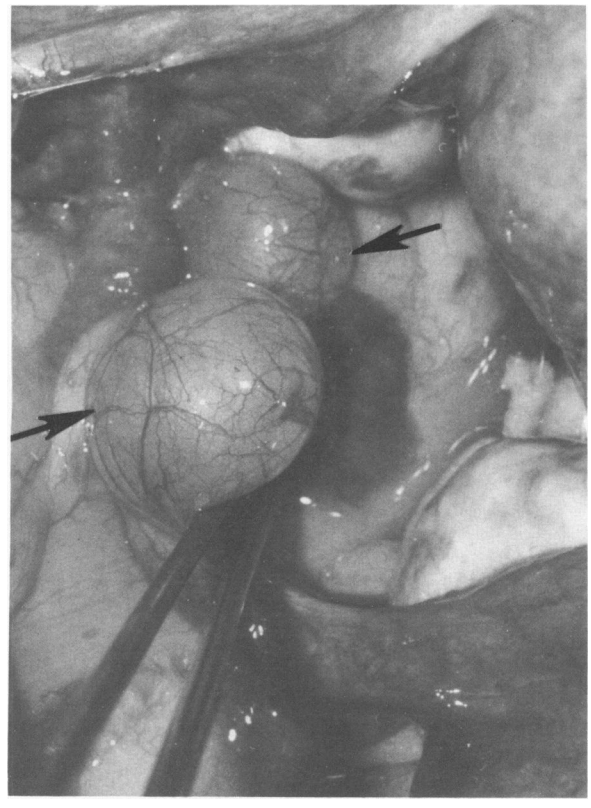

Figure 5—Photograph of the pelvis in Patient 2. Two paraovarian cysts (*arrows*), which are distinct from the ovaries, can be seen. They arise near the fimbriated end of the fallopian tube within the mesosalpinx.

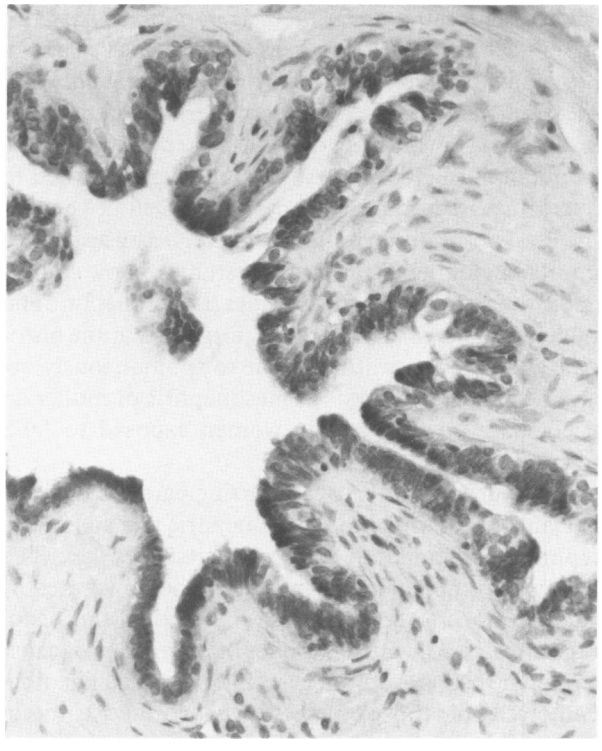

Figure 4—Photomicrograph of the second paraovarian cyst from Patient 1. Note that the cyst wall has a thick fibromuscular wall and the tall columnar epithelium is in a pseudoglandular configuration. (H&E, ×250)

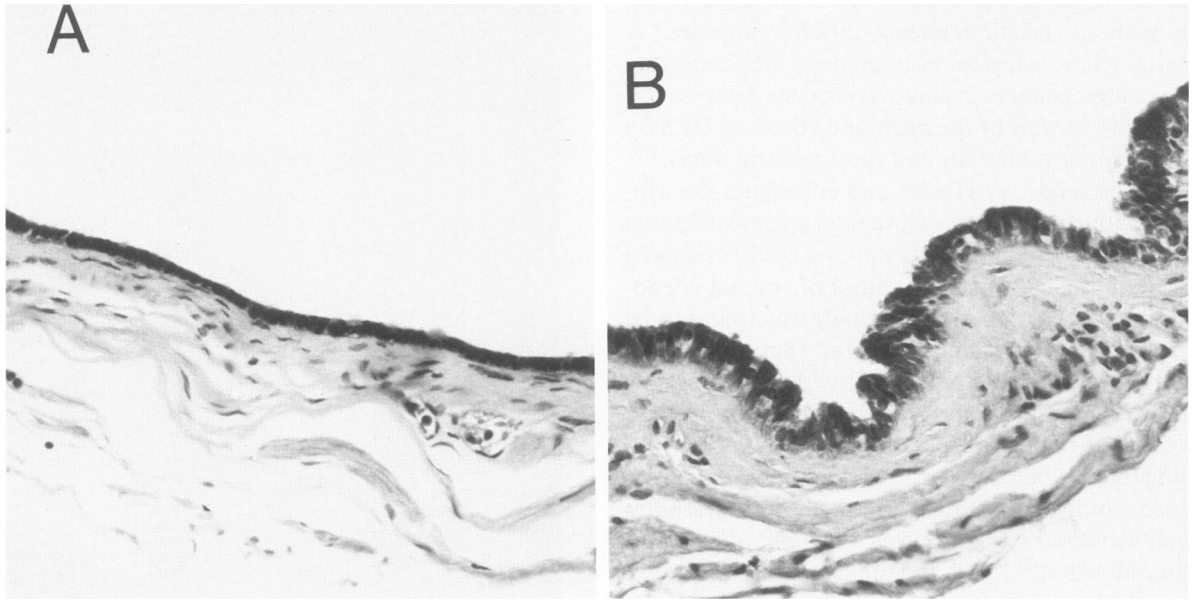

Figure 6—Photomicrographs of the cysts in Patient 2. One of the cysts (**A**) is typical of paraovarian cysts in women not exposed to DES prenatally. The cyst wall is thin and the epithelium is simple cuboidal, probably because of pressure atrophy. By contrast, the second paraovarian cyst (**B**) has tall columnar epithelium and a thickened fibromuscular wall. This is similar in appearance to the nonhemorrhagic cyst in Patient 1. (H&E, ×250)

malities in these paraovarian cysts might be attributable to prenatal DES exposure, analogous to the situation in the mouse.

The frequency of paraovarian cysts in this study was increased in association with prenatal exposure to DES, in comparisons with controls, suggesting a specific effect of DES. A higher frequency of paraovarian cysts associated with prenatal DES exposure is consistent with the findings in mice. In the murine model, paraovarian cysts are rarely observed when the animals are not exposed to DES *in utero*. The probability of a teratogenic effect is probably dependent upon the stage of organogenesis at the time of exposure. Maternal ingestion of DES prior to regression of the mesonephric system in the female embryo would logically be more likely to alter development of that system than exposure later in pregnancy. That cannot be addressed by these data, because all the women were exposed in the first trimester of pregnancy.

It is possible that the fact that these women were infertile may be related to the presence of paraovarian cysts and that fertile DES-exposed women may have a lower frequency of paraovarian cysts. Indeed, 2 of the 5 infertile women with paraovarian cysts excised (patients 2 and 5) conceived within 2 months of their procedures. Although not all the paraovarian cysts had unusual histologic characteristics, the location within the mesosalpinx near the fimbriated end of the fallopian tube was consistent in all the women. This location between the tubal ostia and the ovary has the potential of reducing oocyte retrieval and lowering fecundity. The frequency of infertility in DES-exposed women remains to be determined, and no data exist regarding the frequency of paraovarian cysts in healthy fertile women.

The origin of paraovarian cysts in humans has been the subject of debate over the years. Using criteria such as the microscopic appearance of the epithelium, identification of a basement membrane, the presence or absence of hormonally responsive epithelium, surrounding musculature, etc., several have attempted to determine the origin of the cysts.[23-25] Definitive identification of the origin remains difficult because detailed reports, including ultrastructural studies, show considerable overlap in the characteristics of mesonephric and müllerian-derived tissues.[26] It is possible that the histologic characteristics attributable to spontaneously occurring paraovarian cysts of mesonephric or müllerian origin may not be valid in women exposed to DES prenatally.

Although spontaneously occurring paraovarian cysts probably derive from both mesonephric and paramesonephric duct remnants, the cystic structures described here in the prenatal mouse model are demonstrated by serial sections to be consistent in location and histologic appearance with a mesonephric origin.[6,7] Because several of the paraovarian cysts in the women histologically resemble those cysts in mice identified as mesonephric structures, it is possible the cysts in humans may also be of mesonephric origin. Furthermore, the absence of glycogen is consistent with a nonmüllerian ori-

gin.[27] Of interest, mesonephric duct remnants in the vagina, ie, Gartner's duct cysts, have not been observed with higher frequency in DES-exposed women and were not observed in any of the women reported here. Although no clinical problems have been attributed to the histologic alterations reported here, continued close surveillance would seem prudent in view of the young age of the exposed population and the recent report suggesting that any female adnexal tumor of probable wolffian origin may be potentially malignant even though histologically the mitotic index is low and cellular pleomorphism is lacking.[27]

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